excitatory responses are amplified. Then, excitation mediated by gap junctions can contribute significantly to very precise spike synchronization on the millisecond scale through a mechanism similar to that observed at the electrical synapses between the club endings and the Mauthner cell.

Electrical synapses also mediate a surprising behavior in the Golgi cell network: the arrival of sparse and coincident feed-forward input from mossy fibers can actually desynchronize the Golgi network. This results from the filtering properties and broad interconnectivity afforded by electrical synapses. Spiking input from mossy fibers can induce spikes including an after-hyperpolarization in a Golgi cell directly postsynaptic to it. These spikes, in turn, filtered through electrical synapses, can induce attenuated spikelets but strong hyperpolarizations in neighboring Golgi cells that do not receive direct synaptic input from the spiking mossy fibers. The strong hyperpolarizing component of the gap junction potential then briefly inhibits spike generation in the neighboring cells, desynchronizing the coupled cells.

Synchronization mechanisms in some other cell populations appear to work differently from those of the Golgi cell network. In the hippocampus and neocortex, spikelets carried through electrical synapses between pyramidal cells seem to play an important role in synchronizing these neurons. Electrical synapses in the hippocampus and neocortical areas between pyramidal cells are thought to underlie ultrafast oscillations (~200 Hz), whereas those between interneurons are important for oscillations at gamma frequencies (20~80 Hz). Many interesting questions remain about the existence and roles of gap junctions between pyramidal cells, though. The connexins that underlie the electrical synapses between pyramidal cells remain to be elucidated: ultrafast oscillations are intact in knockout mice lacking connexin 36, in which gamma frequency oscillations are impaired, while pharmacological gap junction blockers abolish high frequency oscillations.

Outlook

Gap junctions and the electrical synapses they form play important and varying roles throughout the brain. Electrical synapses form before chemical synapses and help shape the brain's development. But, throughout life, they filter, distribute, and coordinate neural activity in complex and well-regulated ways that have only recently come into focus. Exploring the computational properties of gap junctions is an active area of research; many questions remain about the diverse contributions they make to establish and regulate neural synchrony. Also, to date, most studies on electrical synapses have focused on connexin 36, which is the most prevalent; but other connexins and pannexins are also expressed in neurons, and their contributions to shaping neural activity in the brain remain largely unknown. New molecular and computational tools will no doubt add to our knowledge of gap junctions and electrical synapses.

Further reading

Bloomfield, S.A., and Völgyi, B. (2009). The diverse functional roles and regulation of neuronal gap junctions in the retina. Nat. Rev. Neurosci. *10*, 495–506.
Dugué, G.P., Brunel, N., Hakim, V., Schwartz, E., Chat, M., Lévesque, M., Courtemanche, R., Léna, C., and Dieudonné, S. (2009). Electrical coupling mediates tunable low-frequency oscillations and resonance in the cerebellar Golgi cell network. Neuron *61*, 126–139.
Haas, J.S., Zavala, B., and Landisman, C.E. (2011). Activity-dependent long-term depression of electrical synapses. Science *334*, 389–393.
Huang, J., Zhang, W., Qiao, W., Hu, A., and Wang, Z. (2010). Functional connectivity and selective odor responses of excitatory local interneurons in Drosophila antennal lobe. Neuron *67*, 1021–1033.
Johnstone, S.R., Billaud, M., Lohman, A.W., Taddeo, E.P., and Isakson, B.E. (2012). Posttranslational modifications in connexins and pannexins. J. Membr. Biol. *245*, 319–332.
Mercer, A. (2012). Electrically coupled excitatory neurones in cortical regions. Brain. Res. *1487*, 192–197.
Montoro, R.J., and Yuste, R. (2004). Gap junctions in developing neocortex: a review. Brain. Res. Brain. Res. Rev. *47*, 216–226.
Pereda, A.E., Curti, S., Hoge, G., Cachope, R., Flores, C.E., and Rash, J.E. (2013). Gap junction-mediated electrical transmission: regulatory mechanisms and plasticity. Biochim. Biophys. Acta. *1828*, 134–146.
Yaksi, E., and Wilson, R.I. (2010). Electrical coupling between olfactory glomeruli. Neuron *67*, 1034–1047.
Yu, Y.C., He, S., Chen, S., Fu, Y., Brown, K.N., Yao, X.H., Ma, J., Gao, K.P., Sosinsky, G.E., Huang, K., and Shi, S.H. (2012). Preferential electrical coupling regulates neocortical lineage-dependent microcircuit assembly. Nature *486*, 113–117.

NIH-NICHD, 35 Lincoln Drive, Room 3A-102, msc 3715, Bethesda, MD 20892, USA.
E-mail: stopferm@mail.nih.gov

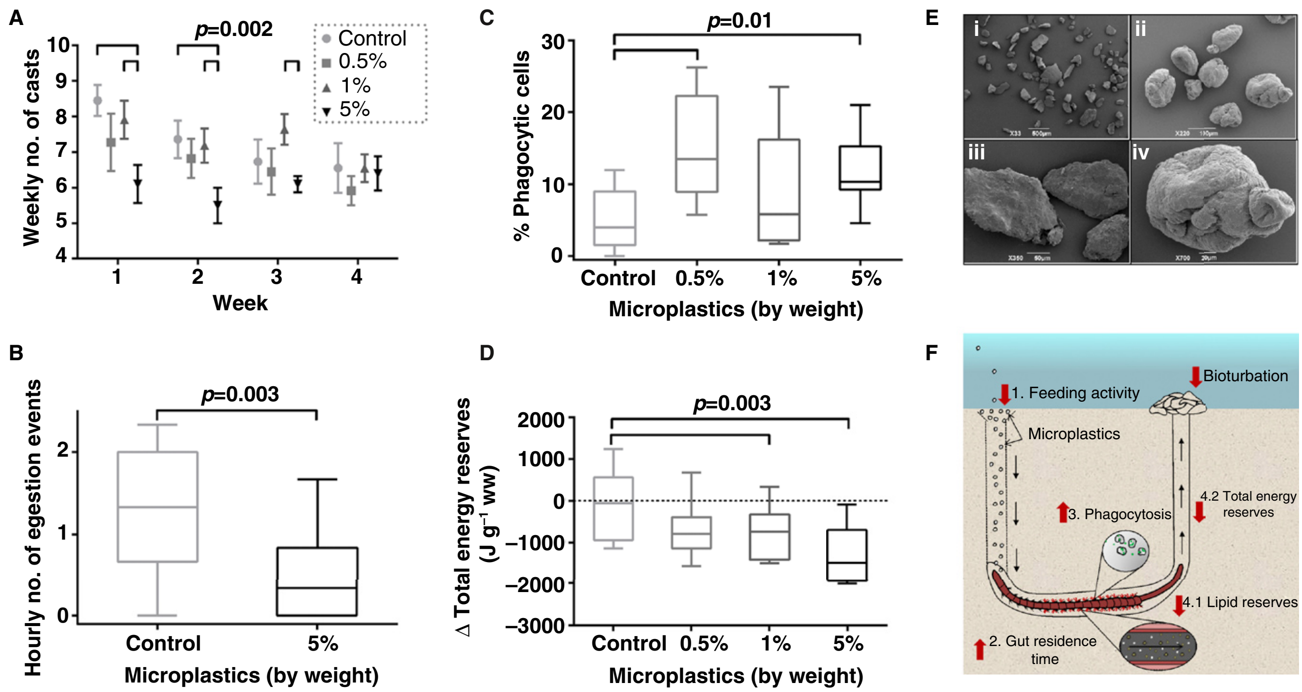
Correspondences

Microplastic ingestion decreases energy reserves in marine worms

Stephanie L. Wright[1], Darren Rowe[1], Richard C. Thompson[2], and Tamara S. Galloway[1,*]

The indiscriminate disposal of plastic to the environment is of concern. Microscopic plastic litter (<5 mm diameter; 'microplastic') is increasing in abundance in the marine environment, originating from the fragmentation of plastic items and from industry and personal-care products [1]. On highly impacted beaches, microplastic concentrations (<1mm) can reach 3% by weight, presenting a global conservation issue [2]. Microplastics are a novel substrate for the adherence of hydrophobic contaminants [1], deposition of eggs [3], and colonization by unique bacterial assemblages [4]. Ingestion by indiscriminate deposit-feeders has been reported, yet physical impacts remain understudied [1]. Here, we show that deposit-feeding marine worms maintained in sediments spiked with microscopic unplasticised polyvinylchloride (UPVC) at concentrations overlapping those in the environment had significantly depleted energy reserves by up to 50% (Figure 1). Our results suggest that depleted energy reserves arise from a combination of reduced feeding activity, longer gut residence times of ingested material and inflammation.

Seabeds worldwide are composed of a range of organic and inorganic sediments that sustain a vast range of marine species. The polychaete worm *Arenicola marina* (lugworm) of the globally distributed family Arenicolidae is a keystone species inhabiting intertidal sediments in Northern Europe; it bioturbates and irrigates the sediment and is an important secondary producer, as a prey species for fish and wading birds. Using a laboratory mesocosm, we performed chronic (four weeks) and short-term (48 hours) experiments, exposing *A. marina* to natural sediments containing clean, chemically-inert UPVC ranging from 0–5% by weight. PVC is denser than



Current Biology

Figure 1. The impacts of microscopic UPVC on *A. marina*.
(A) The effects of UPVC exposure on weekly feeding activity (Generalised Estimating Equation (GEE); p = 0.002 for 'time*treatment'). Data are presented as weekly average (mean ± SE) per worm. (B) The average (median ± range) hourly number of egestion events following 48 h exposure (Mann Whitney U test, p = 0.003). (C) Effects of UPVC exposure on phagocytosis (one-way ANOVA, p = 0.01), which was enhanced for 0.5% and 5% exposed worms (Fisher's LSD test, p = 0.002 and p = 0.013 for 0.5% and 5%, respectively). (D) The effects of UPVC exposure on total available energy reserves in *A. marina*. Data presented as average (median ± range) compared with pre-exposure baseline (dotted line) (one way ANOVA, p=0.003). For 1% and 5% exposed worms, p = 0.036 and 0.001, respectively (Fisher's LSD test). (E) Scanning electron micrographs of i) natural sediment (x33, scale bar 500 µm); ii) UPVC (x220, scale bar 100 µm); iii) natural sediment (x350, scale bar 50 µm); iv) UPVC (x700, scale bar 20 µm). (F) A conceptual model of the effects of microscopic UPVC on *A. marina*: 1) suppressed feeding activity; 2) prolonged gut residence times; 3) inflammation; and 4) reduced lipid and total available energy reserves. Horizontal bars indicate a significant difference at the 0.05 confidence level. Data for the following can be found in Supplemental Information: statistical output; impacts on average feeding activity, cumulative number of casts, feeding status and egestion time; feeding activity in reduced food concentration; grain size distribution of natural sediment and UPVC; differences in weight (pre- and post-exposure); impacts on lipid, protein and sugar reserves.

seawater and sinks out of suspension to sediments; >25% of microplastics sampled from estuarine sediments inhabited by *A. marina* were PVC [5]. Thus, we selected UPVC, mimicking the size and shape of sediment (130 µm mean diameter; Figure 1E). We assessed chronic effects on feeding activity, immunity and energy reserves and made short-term observations on gut residence times.

Worms chronically exposed to 5% UPVC by weight displayed significantly reduced feeding activity compared to control and 1% UPVC-exposed worms (Figure 1A), supporting recent findings whereby 7.4% polystyrene by weight inhibited feeding activity in *A. marina* over 10 days [6]. Reduced feeding activity implies that either fewer particles are ingested overall or a lack of a protein coating on the clean UPVC weakens particle adhesion to the worm's feeding apparatus, reducing uptake efficiency. Suppressed feeding activity may decrease energy assimilation, compromising fitness. It could also decrease bioturbation and therefore oxygenation of the sediment, which is crucial for maintaining infaunal diversity.

Chronic UPVC exposure significantly increased the phagocytic activity of *A. marina*'s immune cells, although this was not dose-dependent (Figure 1C). Enhanced phagocytic activity is indicative of an inflammatory response, which is a metabolically demanding process. Interestingly, the UPVC powder is classified as an irritant to human health following dermal contact.

The total available energy reserves in worms chronically exposed to 1% and 5% UPVC by weight were significantly reduced compared to pre-exposure and control animals. Worms exposed to 5% UPVC by weight had approximately 50% less total available energy reserves compared to controls (Figure 1D) and all UPVC-exposed animals had significantly lower lipid reserves than controls (Supplemental information). Jonker *et al*. [7] found lipid reserves declined in a freshwater oligochaete worm (*Lumbriculus variegatus*) following chronic exposure to powdered activated carbon, presumably due to reduced feeding activity. In our experiments, depleted energy reserves, which closely followed the trend for lipid reserves, could compromise somatic maintenance and growth, maturity and reproduction.

We determined the time it took ingested material to traverse the gut and found intervals between egestion events were 1.5 times longer (mean 1086 seconds) in animals exposed to 5% UPVC by weight, with an average of 0.33 ± 1 (median ± range) hourly egestion events compared to control worms (1.33 ± 2.33 (median ± range); Figure 1B; Supplemental information). *A. marina*'s digestion is characterised by material continuously entering and exiting the digestive tract, with negligible mixing during passage. Prolonged gut residence times imply that microplastics, which are of low nutritional value, are being retained and subjected to extensive digestion, at an energetic cost.

Polychaete worms exhibit positive correlations between organic content and feeding activity [8]. We therefore tested the hypothesis that UPVC reduced the organic content of the sediment to a level at which food concentration becomes a limiting factor. When *A. marina* was exposed to natural sediment of varying organic content — altered by adding clean silica sand — there was no significant reduction in feeding activity (Supplemental information). This suggests that the observed reduction in feeding activity of 5% UPVC-exposed worms is likely attributed to a characteristic of the UPVC and not the secondary effect of decreased food concentration.

At a density of 85 individuals per m^2, which is typical of a tidal flat habitat, *A. marina* is estimated to process 400 cm^3 of sediment annually [9]. Microplastic debris (<1 mm) comprising 3.17% by weight of the sediment has been reported, which when adjusted for density could represent up to 6.34% of the sediment volume at contamination hotspots [2]. Using the Wadden Sea, where *A. marina* is a keystone species, as an example, if contamination accumulated to such levels *in situ*, *A. marina* could consume up to 33 m^3 of microplastics annually. We found overall feeding activity reduced by approximately 25% in worms exposed to 5% UPVC by weight for a month. Using the Wadden Sea example, this would result in 130 m^3 less sediment being reworked annually. Our current observations indicate that 1% microplastics by weight can reduce total energy reserves by approximately 30%, mainly linked to a reduction in lipid reserves. We propose a conceptual model (Figure 1F) whereby high concentrations of microplastics could induce suppressed feeding activity, prolonged gut residence times, inflammation and reduced energy reserves, impacting on growth, reproduction and ultimately survival. We have shown that microplastics can cause physical harm to an important marine species, emphasizing the need to reconsider how discarded PVC, polystyrene, polyurethane and polycarbonate (30% of global production) are classified in terms of hazard [10].

Supplemental Information

Supplemental Information including experimental procedures and two figures can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.10.068.

Acknowledgments

This work was funded by the Department for Environment, Food & Rural Affairs; 1-SW-P-N21-000-031-DN-A1-05102. We thank Peter Splatt for SEM imaging assistance, Professor Stuart Bearhop for invaluable comments on the manuscript and Dr. Adil Bakir for UPVC chemistry analyses.

References

1. Wright, S.L., Thompson, R.C., and Galloway, T.S. (2013). The physical impacts of microplastics on marine organisms: A review. Environ. Poll. *178*, 483–492.
2. Carson, H.S., Colbert, S.L., Kaylor, M.J., and McDermid, K.J. (2011). Small plastic debris changes water movement and heat transfer through beach sediments. Mar. Pollut. Bull. *62*, 1708–1713.
3. Goldstein, M.C., Rosenberg, M., and Cheng, L. (2012). Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect. Biol. Lett. *8*, 817–820.
4. Zettler, E.R., Mincer, T.J., and Amaral-Zettler, L.A. (2013). Life in the "plastisphere": microbial communities on plastic marine debris. Environ. Sci. Technol. *47*, 7137–7146.
5. Browne, M.A., Galloway, T.S., and Thompson, R.C. (2010). Spatial patterns of plastic debris along estuarine shorelines. Environ. Sci. Technol. *44*, 3404–3409.
6. Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., and Koelmans, A.A. (2012). Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). Environ. Sci. Technol. *47*, 593–600.
7. Jonker, M.T.O., Suijkerbuijk, M.P.W., Schmitt, H., and Sinnige, T.L. (2009). Ecotoxicological effects of activated carbon addition to sediments. Environ. Sci. Technol. *43*, 5959–5966.
8. Hymel, S.N., and Plante, C.J. (2000). Feeding and bacteriolytic responses of the deposit-feeder Abarenicola pacifica (Polychaeta: Arenicolidae) to changes in temperature and sediment food concentration. Mar. Biol. *136*, 1019–1027.
9. Cadée, G.C. (1976). Sediment reworking by arenicola marina on tidal flats in the Dutch Wadden Sea. Netherlands J. Sea Res. *10*, 440–460.
10. Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S., and Thompson, R.C. (2013). Policy: Classify plastic waste as hazardous. Nature *494*, 169–171.

[1]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, Devon EX4 4QD, UK. [2]School of Marine Science and Engineering, University of Plymouth, Plymouth, Devon PL4 8AA, UK.
*E-mail: T.S.Galloway@exeter.ac.uk

Evidence of episodic-like memory in cuttlefish

Christelle Jozet-Alves[1,2], Marion Bertin[1,2], and Nicola S. Clayton[3]

The recollection of past experiences allows us to recall what happened during a particular event, and where and when it occurred [1]. Since the first study on episodic-like memory in scrub-jays [2], there has been widespread acceptance of the idea that tests in animals should integrate the 'what', 'where' and 'when' components of a unique event that occurred in the past [3,4]. This is referred to as episodic-like memory rather than episodic memory *per se*, in acknowledgement of the lack of evidence for, or against, the phenomenological aspects that accompany episodic recollection in humans. So far, evidence for episodic-like memory has only been found in some birds and mammals. We show here that cuttlefish, cephalopod mollusks, keep track of what they have eaten, and where and how long ago they ate, in order to match their foraging behavior with the time of replenishing of different foods. Foraging in cuttlefish fulfils the criteria of 'what', 'where' and 'when' of unique events and thus provides behavioral evidence of episodic-like memory in an invertebrate.

Cuttlefish rest more than 95% of the time: long rest periods in safety are punctuated by brief foraging bouts in the open [5]. They appear to minimize their hunting time, and then quickly return to safe places. The common cuttlefish *Sepia officinalis* has good visual discrimination and spatial abilities [6], and displays strong food preferences [7]. We therefore hypothesized that cuttlefish may keep track of what they have eaten, and where and how long ago they ate, in order to match their foraging behavior with the time at which different foods replenish, a feature we shall refer to as 'replenishing rate'.

We first conducted tests to determine the feeding preferences of each cuttlefish, by giving them the choice between a crab and a shrimp (10 times per cuttlefish over